FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
217415US0PCT	10/019439

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR00/01857	30 JUNE 2000	01 JULY 1999

TITLE OF INVENTION

FIBRIN CITRULLINE DERIVATIVES AND THEIR USE FOR DIAGNOSING OR TREATING RHEUMATOID ARTHRITIS

APPLICANT(S) FOR DO/EO/US

Guy SERRE, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Notice of Priority
PTO-1449
Drawings (3 sheets)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CER)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/019439	PCT/FR00/01857	217415US0PCT

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1040.00
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$890.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$740.00
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$710.00
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT = **$890.00**

Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)). **$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	10 - 20 =	0	x $18.00	$0.00
Independent claims	1 - 3 =	0	x $84.00	$0.00
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00

TOTAL OF ABOVE CALCULATIONS = **$1,020.00**

☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2. **$0.00**

SUBTOTAL = **$1,020.00**

Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). + **$0.00**

TOTAL NATIONAL FEE = **$1,020.00**

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐ **$0.00**

TOTAL FEES ENCLOSED = **$1,020.00**

Amount to be: refunded	$
charged	$

a. ☒ A check in the amount of **$1,020.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

22850

(703) 413-3000

Surinder Sachar
Registration No. 34,423

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

Dec. 31 2001
DATE

APPLICATION DATA SHEET

APPLICATION INFORMATION

Application Number::	10/019,439
Application Date::	12/31/01
Application Type::	REGULAR
Subject Matter::	UTILITY
CD-ROM or CD-R?::	NONE
Title::	FIBRIN CITRULLINE DERIVATIVES AND THEIR USE FOR DIAGNOSING OR TREATING RHEUMATOID ARTHRITIS
Attorney Docket Number::	217415US0PCT

INVENTOR INFORMATION

Applicant Authority Type::	INVENTOR
Primary Citizenship Country::	France
Status::	FULL CAPACITY
Given Name::	Guy
Family Name::	SERRE
City of Residence::	Toulouse
Country of Residence::	France
Street of Mailing Address::	Residence du Lac, Appartement 46, 10, avenue Winston Churchill
City of Mailing Address::	Toulouse
Country of Mailing Address::	France
Postal or Zip Code of Mailing Address::	F-31100

Applicant Authority Type::	INVENTOR
Primary Citizenship Country::	France
Status::	FULL CAPACITY
Given Name::	Mireille
Family Name::	SEBBAG
City of Residence::	Toulouse
Country of Residence::	France
Street of Mailing Address::	3, rue A. Fredeau
City of Mailing Address::	Toulouse
Country of Mailing Address::	France
Postal or Zip Code of Mailing Address::	F-31500

CORRESPONDENCE INFORMATION

Correspondence Customer Number::	22850

REPRESENTATIVE INFORMATION

Representative Customer Number:: 22850

DOMESTIC PRIORITY INFORMATION

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	National Stage of	PCT/FR00/01857	06/30/00

FOREIGN PRIORITY INFORMATION

Application Number:	Country::	Filing Date::	Priority Claimed::
99/08470	France	07/01/99	YES

ASSIGNMENT INFORMATION

Assignee Name::	UNIVERSITE PAUL SABATIER-TOULOUSE III
Street of Mailing Address::	118, route de Narbonne
City of Mailing Address::	Toulouse Cedex 4
Country of Mailing Address::	France
Postal or Zip Code of Mailing Address::	F-31062

217415US-0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF:	:
GUY SERRE ET AL.	:
SERIAL NO: NEW U.S. PCT APPLN. (Based on PCT/FR00/01857)	: ATTN: APPLICATION BRANCH
FILED: HEREWITH	:
FOR: FIBRIN CITRULLINE DERIVATIVES AND THEIR USE FOR DIAGNOSING OR TREATING RHEUMATOID ARTHRITIS	

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows.

IN THE CLAIMS

Please amend the claims as shown on the marked-up following this amendment to read as follows.

3. (Amended) The citrullinated polypeptide as claimed in claim 1, wherein said vertebrate fibrin is a mammalian fibrin, preferably a human fibrin.

4. (Amended) The use of a polypeptide as claimed in claim 1, for diagnosing rheumatoid arthritis, *in vitro*.

5. (Amended) An antigenic composition for diagnosing the presence of rheumatoid arthritis-specific autoantibodies in a biological sample, characterized in that it contains at least one citrullinated polypeptide as claimed in claim 1, optionally labeled with and/or conjugated to a carrier molecule.

6. (Amended) A method for detecting rheumatoid arthritis specific autoantibodies in a biological sample, which method is characterized in that it comprises:

bringing said biological sample into contact with at least one polypeptide as claimed in claim 1, under conditions which allow the formation of an antigen/antibody complex with the rheumatoid arthritis-specific autoantibodies possibly present;

detecting, by any suitable means, the antigen/antibody complex possibly formed.

7. (Amended) A kit for detecting rheumatoid arthritis-specific autoantibodies in a biological sample, characterized in that it comprises at least one polypeptide as claimed in claim 1, and also buffers and reagents suitable for constituting a reaction medium which allows the formation of an antigen/antibody complex, and/or means for detecting said antigen/antibody complex.

8. (Amended) The use of a citrullinated polypeptide as claimed in claim 1, for producing a medicinal product.

10. (Amended) A pharmaceutical composition, characterized in that it contains, as active principle, at least one citrullinated polypeptide as claimed in claim 1.

REMARKS

Claims 1-10 are active in the present application. Claims 3-8 and 10 have been amended to remove multiple dependencies. No new matter is added. An action on the merits and allowance of claims is solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon
Attorney of Record
Registration No. 24,618

Stefan U. Koschmieder, Ph.D.
Registration No. 50,238

22850

(703) 413-3000
NFO/DJP/smi

I:\atty\SUKOS\217415US-pr.wpd

Marked-Up Copy
Serial No:

Amendment Filed on:
12-31-01

IN THE CLAIMS

Please amend the claims as follows.

--3. (Amended) The citrullinated polypeptide as claimed in [either of claims 1 and 2, characterized in that] claim 1, wherein said vertebrate fibrin is a mammalian fibrin, preferably a human fibrin.

4. (Amended) The use of a polypeptide as claimed in [any one of claims 1 to 3] claim 1, for diagnosing rheumatoid arthritis, *in vitro*.

5. (Amended) An antigenic composition for diagnosing the presence of rheumatoid arthritis-specific autoantibodies in a biological sample, characterized in that it contains at least one citrullinated polypeptide as claimed in [any one of claims 1 to 3] claim 1, optionally labeled with and/or conjugated to a carrier molecule.

6. (Amended) A method for detecting rheumatoid arthritis specific autoantibodies in a biological sample, which method is characterized in that it comprises:

bringing said biological sample into contact with at least one polypeptide as claimed in [any one of claims 1 to 3] claim 1, under conditions which allow the formation of an antigen/antibody complex with the rheumatoid arthritis-specific autoantibodies possibly present;

detecting, by any suitable means, the antigen/antibody complex possibly formed.

7. (Amended) A kit for detecting rheumatoid arthritis-specific autoantibodies in a biological sample, characterized in that it comprises at least one polypeptide as claimed in [any one of claims 1 to 3] claim 1, and also buffers and reagents suitable for constituting a reaction medium which allows the formation of an antigen/antibody complex, and/or means for detecting said antigen/antibody complex.

8. (Amended) The use of a citrullinated polypeptide as claimed in [any one of claims 1 to 3] claim 1, for producing a medicinal product.

10. (Amended) A pharmaceutical composition, characterized in that it contains, as active principle, at least one citrullinated polypeptide as claimed in [any one of claims 1 to 3] claim 1.--

FIBRIN CITRULLINE DERIVATIVES AND THEIR USE FOR DIAGNOSING OR TREATING RHEUMATOID ARTHRITIS

The present invention relates to citrullinated derivatives of fibrin and to their uses in diagnosing and treating rheumatoid arthritis.

Rheumatoid arthritis (hereinafter abbreviated to "RA") is the most common of the forms of chronic inflammatory rheumatism. It is an autoimmune disease; the serum of affected patients contains autoantibodies, some of which are specific and may constitute a marker for this disease, allowing it to be diagnosed even at early stages.

Prior studies by the team of the inventors have shown that these antibodies recognize different molecular forms of the (pro)filaggrin family (for review, cf. for example SERRE and VINCENT, In: Autoantibodies, PETER and SHOENFIELD Eds, Elsevier Science Publishers, 271-276, 1996). These antibodies have, for this reason, been named: "antifilaggrin autoantibodies (AFAs)". Application EP 0 511 116 describes the purification and characterization of antigens of the filaggrin family, recognized by these antibodies, and their use for diagnosing rheumatoid arthritis.

The inventors have shown that the epitopes recognized by the AFAs are carried by regions of the filaggrin molecule, in which at least some of the arginines are deiminated and thus transformed into citrulline; citrullinated peptides specifically recognized by AFAs have thus been obtained from the main immunoreactive regions of filaggrin. These peptides, and their use for diagnosing RA, are the subject of Application PCT/FR97/01541 and of Application PCT/FR98/02899 in the name of BIOMERIEUX. The inventors' observations concerning the role of citrulline residues in the reactivity of filaggrin with RA-specific autoantibodies

have subsequently been confirmed by other researchers [SCHELLEKENS et al., Arthritis Rheum., 40, no. 9 supplement, p. S276, summary 1471 (1997); VISSER et al., Arthritis Rheum., 40, no. 9 supplement, p. S289, summary 1551 (1997)].

The inventors have also shown that AFAs represent a considerable proportion of the interstitial immunoglobulins of synovial rheumatoid tissues and that they are synthesized locally by specific plasmocytes present in these tissues, which confirms the hypothesis that they are involved in the autoimmune response associated with RA. The use of filaggrin, or of citrullinated peptides derived therefrom, to neutralize this autoimmune response is the subject of Application PCT/FR98/02900 in the name of UNIVERSITÉ PAUL SABATIER [Paul Sabatier University] (TOULOUSE III).

However, the involvement of filaggrin as an immunogen or as a target antigen in the autoimmune response associated with RA has never been noted. The true antigen involved in this response remains to be identified.

The inventors have now succeeded in characterizing this antigen and have thus shown that it is composed of citrullinated derivatives of the α- and/or β-chains of fibrin.

A subject of the present invention is a citrullinated polypeptide derived from all or part of the sequence of the α-chain or of the β-chain of a vertebrate fibrin, by substitution of at least one arginine residue with a citrulline residue.

Preferably, a polypeptide in accordance with the invention comprises at least 5 consecutive amino acids and advantageously at least 10 consecutive amino acids,

including at least one citrulline, of the sequence of the α-chain or of the β-chain of a mammalian fibrin. Advantageously, said vertebrate fibrin is a mammalian fibrin, preferably a human fibrin.

Citrullinated polypeptides in accordance with the invention may, for example, be obtained from natural, recombinant or synthetic fibrin or fibrinogen, or from fragments thereof, comprising at least one arginine residue, by the action of peptidyl arginine deiminase (PAD); they may also be obtained by peptide synthesis, directly incorporating one or more citrulline residues into the synthesized peptide.

Citrullinated polypeptides in accordance with the invention may also be pseudopeptides having the same three-dimensional structure, and therefore the same immunological reactivity, as the citrullinated polypeptides derived from the α- or β-chains of fibrin, or from fragments thereof, mentioned above. They may, for example, be pseudopeptides of the *retro* type, in which L-amino acids are linked together according to a reverse sequence of that of the peptide to be reproduced, or pseudopeptides of the *retro-inverso* type, consisting of D-series amino acids (instead of the L-series amino acids of natural peptides) linked together according to a reverse sequence of that of the peptide to be reproduced, or alternatively pseudopeptides containing a CH_2-NH bond in place of a CO-NH peptide bond. Pseudopeptides of these various types are, for example, described by BENKIRANE et al. [J. Biol. Chem., 270, p. 11921-11926, (1995); J. Biol. Chem., 271, p. 33218-33224, (1996)]; BRIAND et al. [(J. Biol. Chem., 270, p. 20686-20691, (1995); GUICHARD et al. [J. Biol. Chem., 270, p. 26057-26059, (1995)].

A subject of the present invention is also the use of the polypeptides in accordance with the invention, as defined above, for diagnosing RA, *in vitro*.

The present invention in particular encompasses antigenic compositions for diagnosing the presence of RA-specific autoantibodies in a biological sample, which compositions are characterized in that they contain at least one polypeptide in accordance with the invention, optionally labeled with and/or conjugated to a carrier molecule.

A subject of the present invention is also a method for detecting RA-specific autoantibodies of the G class in a biological sample, which method is characterized in that it comprises:

- bringing said biological sample into contact with at least one polypeptide in accordance with the invention, as defined above, under conditions which allow the formation of an antigen/antibody complex with the RA-specific autoantibodies possibly present;

- detecting, by any suitable means, the antigen/antibody complex possibly formed.

This detection method may be carried out using a kit comprising at least one antigen according to the invention, and also buffers and reagents suitable for constituting a reaction medium which allows the formation of an antigen/antibody complex, and/or means for detecting said antigen/antibody complex.

Said kit may also comprise, where appropriate, reference samples, such as one or more negative serum (sera) and one or more positive serum (sera).

A subject of the present invention is also the use of citrullinated polypeptides in accordance with the invention, for producing a medicinal product, and especially a medicinal product intended to neutralize the autoimmune response associated with RA, and in particular to inhibit the attachment of the humoral or cellular effectors of this autoimmune response, to the citrullinated derivatives of α- or β-chains of fibrin which are present in rheumatoid tissues.

This *in vivo* neutralization of the autoimmune response may contribute to treating RA or other diseases which are thought to involve lesions induced by an autoimmune response directed against epitopes exhibiting cross-reactions with the citrullinated derivatives of α- or β-chains of fibrin.

Advantageously, for *in vivo* administration, polypeptides modified so as to prolong their lifetime in the organism, in particular by increasing their resistance to proteases, will be chosen; they may in particular be pseudopeptides, such as those mentioned above.

The present invention also encompasses pharmaceutical compositions, in particular for treating rheumatoid arthritis, characterized in that they contain, as active principle, at least one polypeptide in accordance with the invention.

Pharmaceutical compositions in accordance with the invention may be administered by any suitable means known per se. They may, for example, be administered systemically, orally, parenterally, or by subcutaneous, intravenous or intramuscular injection; they may also be administered locally, for example by intra-articular injections or by microinjections, under arthroscopy, into the inflammatory synovial tissue.

The present invention will be more clearly understood using the additional description which follows, which refers to the identification of deiminated forms of the α-chain or β-chain of human fibrin in rheumatoid tissues, and to the use of deiminated fibrinogen for detecting the presence of AFAs in serum samples.

EXAMPLE 1: PURIFICATION AND CHARACTERIZATION OF ANTIGENIC PROTEINS RECOGNIZED BY AFAs IN RHEUMATOID SYNOVIAL TISSUES

1) Analysis of rheumatoid synovial tissues

Materials and methods:

The synovial tissue samples used for the protein extractions were taken from patients suffering from rheumatoid arthritis, during a synovectomy or an arthroplasty of the wrist or knee, and all correspond to tissue fragments which are the seat of conventional histological rheumatoid synovitis lesions. They are conserved by freezing in isopentane cooled with liquid nitrogen.

Synovial tissue fragments originating from four patients were extracted sequentially, in a low ionic strength buffer, a urea buffer and in a urea/DTT buffer, successively.

Preparation of synovial extracts

The extraction was carried out using an Ultra-Turrax homogenizer (T25 basic, IKA Labortechnik, Staufen, Germany) with a volume of 6 ml of buffer per gram of tissue.

The following buffers were used at a temperature of 0°C: 40 mM Tris-HCl, pH 7.4, containing 150 mM of NaCl [low

ionic strength buffer]; 40 mM Tris-HCl, pH 7.4, containing 8M urea deionized on an ion exchange resin (AG 501-X8, Biorad, Hercules, CA) [urea buffer]; 40 mM Tris-HCl, pH 7.4, containing 8M deionized urea and 50 mM dithiothreitol (DTT), (Sigma) [urea/DTT buffer]. All the buffers were supplemented with 20 mM EDTA, 0.02% sodium azide, 2 µg/ml aprotinin, 10 mM N-ethylmaleimide and 1 mM phenylmethylsulfonyl fluoride (Sigma, Saint Louis, MI). After each extraction, the homogenates were centrifuged for 20 minutes at 15,000 g, at the temperature of 4°C. The urea buffer and urea/DTT buffer extracts were dialyzed against water before being analyzed by electrophoresis and by immunotransfer.

Electrophoresis and immunodetection

The synovial proteins of the various extracts were separated by electrophoresis on a 10% polyacrylamide gel in denaturing SDS buffer (SDS-PAGE), and were then electrotransferred onto reinforced nitrocellulose membranes (Hybond-™C extra, Amersham, Little Chalfont, UK).

The membranes were immunodetected with the following antibody preparations; AFA-positive or AFA-negative rheumatoid human sera; non-rheumatoid control human sera derived from patients suffering from other forms of inflammatory rheumatism or from healthy individuals (1/100); purified fractions of AFAs (10 µg/ml); mouse monoclonal antibody directed against human fibrin and fibrinogen (5 µg/ml); two sheep antisera directed, respectively, against recombinant α- and γ-chains of human fibrinogen (1/1000) (Cambio, Cambridge, UK); a rabbit antiserum directed against the recombinant β-chain of human fibrinogen (1/200000) (Cambio).

The human sera used are derived from 95 patients suffering from rheumatoid arthritis (RA), perfectly characterized from a clinical and biological point of view according to the criteria of the American College of Rheumatology, from 24 patients suffering from non-rheumatoid inflammatory rheumatism or from non-inflammatory pathological conditions (control sera) and from 10 healthy individuals. The semi-quantitative titration of the antifilaggrin antibodies (AFAs) in the sera was carried out by indirect imunofluorescence on cryosections of rat esophageal epithelium and by immunotransfer on epidermal extracts enriched in filaggrin acid variant, according to previously published protocols [VINCENT et al., Ann. Rheum. Dis., 48, 712-722 (1989); VINCENT et al., J. Rheumatol., 25, 838-846 (1998)]. The "AFA-positive" sera are those which exhibit AFAs at significant titers after detection using both methods, and the "AFA-negative" sera are those which do not exhibit detectable AFAs by either of the two methods.

The AFAs were purified by affinity chromatography on the epidermal filaggrin acid variant, according to the protocol described by GIRBAL-NEUHAUSER et al. (J. Immunol., 162, 585-594 (1999), using 45 rheumatoid sera having a high AFA titer. The purified antibody fractions were pooled.

Peroxidase-conjugated secondary molecular probes were used for detecting all the primary antibodies: protein A (Sigma), sheep antibodies directed against mouse IgGs (Biosys, Compiègne, France), goat Fab fragments directed against rabbit IgGs (Biosys) and rabbit F(ab')2 fragments directed against sheet IgGs (Southern Biotech. Inc), for detecting, respectively, human, murine, rabbit and sheep IgGs. The peroxidase activity was visualized using the ECL™ detection system

(Amersham International, Aylesbury, UK), according to the protocol provided by the manufacturer.

Results

Specific reactivity with the purified AFAs and the AFA-positive rheumatoid sera was observed only in the extract produced in urea/DTT buffer.

The results are illustrated by figure 1:

Legend to figure 1:

- AFAp = purified AFAs;
- RA sera = rheumatoid sera:
  - * AFA+ = AFA-positive;
  - * AFA- = AFA-negative;
- control sera = sera derived from patients suffering from forms of inflammatory rheumatism other than RA, or from healthy donors.

These results show that the specific reactivity with the purified AFAs and the AFA-positive rheumatoid sera relates to two protein bands of apparent molecular weight of approximately 64 kD to approximately 78 kD (w64-78) and of approximately 55 kD to approximately 61 kD (w55-61), respectively. These protein bands were not detected by the AFA-negative sera, regardless of whether they originate from patients suffering from RA or from other forms of inflammatory rheumatism, or are derived from healthy donors.

The presence of these proteins specifically recognized by the purified AFAs and the AFA-positive rheumatoid sera was observed in the urea/DTT extracts of synovial tissues derived from the 4 rheumatoid patients studied.

In total, 48 AFA-positive rheumatoid sera were tested by immunotransfer on at least one synovial urea/DTT

extract. Among the sera, 40 recognized w64-78, 39 recognized w55-61, 37 recognized both w64-78 and w55-61, 3 recognized only w64-78 and 2 recognised only w55-61.

Thirteen AFA-negative rheumatoid sera were tested by immunotransfer on at least one urea/DTT extract of synovial tissue; none of these sera recognized either w64-78 or w55-61.

Ten sera derived from healthy donors and 5 sera derived from patients suffering from other forms of inflammatory rheumatism were also tested by immunotransfer on at least one synovial urea/DTT extract; none of these sera recognized either w64-78 or w55-61.

2) Characterization of the w64-78 and w55-61 antigenic proteins

The proteins of the urea/DTT buffer extract of the synovial tissue of one of the patients suffering from RA were precipitated with 4 volumes of glacial acetone and then redissolved in the urea/DTT buffer at a concentration 15 times higher than their initial concentration.

The proteins of the concentrated extract were separated by two-dimensional electrophoresis, by isoelectrofocussing followed by SDS-PAGE.

A two-dimensional electrophoretic separation was carried out in the PhastSystem™ (Pharmacia). The first electrophoretic separation was performed on PhastGel™ isoelectrofocussing (IEF) gels which, beforehand, had been washed, dried and rehydrated in a deionized buffer containing 8 M urea, 0.5% Nonidet P-40 and ampholytes creating a pH gradient of 3 to 10 (Pharmacia). The

second dimension was performed by SDS-PAGE on 7.5% polyacrylamide gels.

The proteins were then electrotransferred onto polyvinyl difluoride (PVDF) membranes (ProBlott™ membranes, Applied Biosystems, Foster City, CA), in 50 mM Tris and 50 mM of boric acid. The membranes were finally stained with an aqueous solution of amido black at 0.1%, of acetic acid at 1% and of methanol at 45%, or immunodetected with rheumatoid sera according to the protocol described in 1) above.

Figure 2 illustrates the profiles obtained after electrotransfer onto a PVDF membrane and:

a) staining with amido black; or
b) immunodetection with an AFA-positive rheumatoid serum; or
c) immunodetection with an AFA-negative rheumatoid serum.

Legend to figure 2:
- Amido Black = staining with amido black;
- AFA+ = immunodetection with an AFA-positive rheumatoid serum;
- AFA- = immunodetection with an AFA-negative rheumatoid serum.

After staining with amido black, the presence of two major proteins, with an apparent molecular weight of 64-78 kD and 55-61 kD and pI of approximately 5.85 to approximately 8.45, is observed.

These proteins are immunodetected with the AFA-positive rheumatoid sera but not with the AFA-negative rheumatoid sera.

Using identical transfers onto a PVDF membrane after two-dimensional electrophoresis, membrane fragments corresponding to the center of each immunoreactive zone were excised and then subjected to amino-terminal sequencing in an Applied Biosystems sequencer (494A or 473A), according to the method recommended by the manufacturer.

The sequence gly-pro-arg-val-val-glu-arg-his-gln-ser-ala was obtained from the membrane fragment corresponding to the w64-78 antigen. This sequence is strictly identical to the sequence 36-46 of the product of the human fibrinogen α-chain precursor gene. When membrane fragments corresponding to the right or left ends of the w64-78 immunoreactive zone were excised and then each subjected to three cycles of amino-terminal sequencing, gly-pro-arg sequences were found each time, indicating that the entire p64-78 immunoreactive zone has the same amino-terminal end.

The sequence gly-his-arg-pro-leu-asp-lys-lys-arg was obtained from the membrane fragment corresponding to the center of the immunoreactive zone corresponding to the w55-61 antigen. This sequence is strictly identical to the sequence 45-54 of the product of the human fibrinogen β-chain precursor gene. When a membrane fragment corresponding to the left end of the w55-61 immunoreactive zone was excised and then subjected to two cycles of amino-terminal sequencing, the gly-his sequence was found. When a membrane fragment corresponding to the right end of the w55-61 immunoreactive zone was excised and then subjected to six cycles of amino-terminal sequencing, the gly-his-arg-pro-leu-asp sequence and the gly-pro-arg-val-val-glu sequence were found. This indicates that the entire w55-61 immunoreactive zone has the same amino-terminal end and that it partially co-migrates with the w64-78 antigen.

The amino-terminal ends of the w64-78 and w55-61 antigenic proteins correspond, respectively, to the amino-terminal ends of the α- and β-chains of human fibrinogen after respective cleavage, by thrombin, of fibrinopeptides A and B. The amino-terminal ends of the w64-78 and w55-61 antigenic proteins are therefore identical, respectively, to that of the α-chain and to that of the β-chain of human fibrin.

The apparent molecular weights of the w64-78 and w55-61 antigens are compatible with the respective theoretical molecular weight values for the α-chain and for the β-chain of human fibrin.

The identity of the w64-78 antigen and of the α-chain of fibrin, on the one hand, and that of the w55-61 antigen and of the β-chain of fibrin, on the other hand, were confirmed by analyzing the reactivity of antifibrin(ogen) antibodies with respect to these antigens. By immunotransfer, using an extract of synovial tissue prepared in urea/DTT, the "311" mouse monoclonal antibody, which recognizes the three chains α, β and weakly, γ of human fibrinogen and fibrin, is mainly reactive with respect to the w64-78 and w55-61 antigens. Similarly, two antisera, one from sheep and the other from rabbit, directed, respectively, against recombinant α- and β-chains of fibrinogen, recognized mainly a protein which co-migrates with the w64-78 antigen and a protein which co-migrates with the w55-61 antigen, respectively.

EXAMPLE 2: REACTIVITY OF RHEUMATOID SERA AND OF PURIFIED AFAs WITH DEIMINATED FIBRINOGEN *IN VITRO*

The reactivity with respect to deiminated and nondeiminated fibrinogen was studied by immunotransfer. The following were used: the purified AFA fractions, 37

AFA-positive rheumatoid sera of decreasing titer, 10 AFA-negative rheumatoid sera and 19 AFA-negative sera derived from patients suffering from forms of inflammatory or non-inflammatory rheumatism (AFA titers determined by immunotransfer on epidermal extracts enriched in filaggrin acid variant).

The results are illustrated by Figure 3A in the case of nondeiminated fibrinogen and by Figure 3B in the case of deiminated fibrinogen.

Legend to Figure 3:

Figure 3A: non deiminated purified human fibrinogen;

- 311 = antifibrinogen monoclonal antibody 311;
- control sera = sera derived from patients suffering from forms of inflammatory rheumatism other than RA, or from healthy donors;
- RA sera = rheumatoid sera;
  * AFA+ = AFA-positive;
  * AFA- = AFA-negative;

Figure 3B: purified human fibrinogen deiminated with a PAD;

- 311 = antifibrinogen monoclonal antibody 311;
- C1 = sheep antibody directed against mouse IgGs;
- C2 = sheep antibody directed against protein A;
- control sera = sera derived from patients suffering from forms of inflammatory rheumatism other than RA, or from healthy donors;
- RA sera = rheumatoid sera;
  * AFA+ = AFA-positive;
  * AFA- = AFA-negative;

Nondeiminated fibrinogen

After separation by SDS-PAGE, under the conditions described in example 1 above, the nondeiminated

fibrinogen is composed of 3 polypeptides having respective apparent molecular weights 48 kDa, 58 kDa and 69 kDa, corresponding to the expected apparent molecular masses of the α-, β- and γ-polypeptide chains making up the protein (results not given). The "311" antifibrinogen monoclonal antibody strongly recognizes the α- and β-polypeptide chains and very weakly the γ-polypeptide chain (Figure 3A).

Antisera specific for each of the α-, β- and γ-chains of fibrinogen also showed reactivity with respect to the chain against which they were respectively directed (results not shown).

Deimination of the fibrinogen

A peptidyl arginine deiminase (PAD) purified from rabbit skeletal muscle (Sigma, St. Louis, MO) was used. The human fibrinogen (Calbiochem, San Diego, CA) was incubated at the concentration of 0.86 mg/ml, in the presence or absence of PAD (7 U/mg of protein) for 2 h at 50°C, in 0.1 M Tris-HCl buffer, pH 7.4, containing 10 mM of $CaCl_2$ and 5 mM of DTT. These conditions are those which previously made it possible to generate the epitopes on a human recombinant filaggrin, recognized by AFAs [GIRBAL-NEUHAUSER et al., J. Immunol., 162, 585-594 (1999)]. The deimination was then stopped by adding 2% of SDS and heating at 100°C for 3 min.

After deimination for 2 hours, the electrophoretic mobility by SDS-PAGE of the two α- and β-polypeptides became modified and that of the γ-polypeptide remained unchanged. Specifically, the protein corresponding to the α-chain then appeared in the form of a diffuse band of 82 to 95 kDa and was immunodetected by both the

"311" antifibrinogen monoclonal antibody (figure 3B) and the antiserum directed against the α-chain of fibrinogen (results not shown).

The protein corresponding to the β-chain appeared in the form of a well-defined doublet with the molecular weight of 458 kD for the lower band and 60 kD for the upper band, which was not recognized by the "311" antifibrinogen monoclonal antibody (figure 3B) but was immunodetected by the rabbit antiserum directed against the recombinant β-chain of human fibrinogen (results not shown).

No reactivity for the α-chain or for the β-chain is observed with the C1 and C2 antibodies.

Reactivity of the sera

The reactivity of the sera with respect to the α- and β-chains of nondeiminated fibrinogen proved to be zero or very weak and concerned only a few sera rarely occurring, belonging to no particular subgroup.

On the other hand, after deimination, the polypeptides corresponding to the deiminated α- and β-chains react strongly with the purified AFAs (results not shown) and with all of the 37 AFA-positive rheumatoid sera (with the exception of that which has the lowest AFA titer). Moreover, 6 AFA-negative rheumatoid sera out of 10 also clearly recognized the deiminated α- or β-polypeptides: 2 immunodetected the α-polypeptide and the β-polypeptide doublet, 3 others only detected the β-polypeptide doublet, and only 1 immunodetected exclusively the α-polypeptide. On the other hand, with the exception of a serum derived from a patient suffering from Sjögren's syndrome, which was reactive

on the β-polypeptide doublet, none of the control sera immunodetected the deiminated fibrinogen.

The affinity of the AFA-positive rheumatoid sera with respect to the two deiminated α- and β-polypeptides proved to be slightly variable from one serum to the other. Thus, 6 sera, while strongly detecting the β-polypeptide, only very weakly recognized the α-polypeptide. Similarly, 3 sera, highly reactive with respect to the α-polypeptide, did not detect the deiminated β-polypeptide. Moreover, the intensity of labeling of the two polypeptides appears, overall, to be proportional to the AFA titer of the sera. It should be noted that the sera reactive on the deiminated α and β-polypeptides of fibrinogen were also reactive with respect to high molecular weight (greater than 200 kD) polypeptides generated during the deimination of the fibrinogen. These polypeptides which clearly react with the antifibrinogen antibodies are very probably fibrinogen chain aggregates.

In conclusion, recognition of the α- and β-polypeptides of fibrinogen by rheumatoid sera is not only entirely dependent on their deimination, since the nondeiminated polypeptides are never recognized, but it is also clearly linked to the antifilaggrin reactivity of these sera. It should be noted that these deiminated polypeptides make it possible to detect with great sensitivity the AFAs present in rheumatoid sera.

These results clearly demonstrate that the antigenic targets of the ASAs in rheumatoid synovial joints are deiminated forms of the α-chain and of the β-chain of human fibrin.

CLAIMS

1. A citrullinated polypeptide derived from all or part of the sequence of the α-chain or of the β-chain of a vertebrate fibrin, by substitution of at least one arginine residue with a citrulline residue.

2. The citrullinated polypeptide as claimed in claim 1, derived from a sequence of at least 5 consecutive amino acids of the α-chain or of the β-chain of a vertebrate fibrin.

3. The citrullinated polypeptide as claimed in either of claims 1 and 2, characterized in that said vertebrate fibrin is a mammalian fibrin, preferably a human fibrin.

4. The use of a polypeptide as claimed in any one of claims 1 to 3, for diagnosing rheumatoid arthritis, *in vitro*.

5. An antigenic composition for diagnosing the presence of rheumatoid arthritis-specific autoantibodies in a biological sample, characterized in that it contains at least one citrullinated polypeptide as claimed in any one of claims 1 to 3, optionally labeled with and/or conjugated to a carrier molecule.

6. A method for detecting rheumatoid arthritis-specific autoantibodies in a biological sample, which method is characterized in that it comprises:
- bringing said biological sample into contact with at least one polypeptide as claimed in any one of claims 1 to 3, under conditions which allow

the formation of an antigen/antibody complex with the rheumatoid arthritis-specific autoantibodies possibly present;

- detecting, by any suitable means, the antigen/antibody complex possibly formed.

7. A kit for detecting rheumatoid arthritis-specific autoantibodies in a biological sample, characterized in that it comprises at least one polypeptide as claimed in any one of claims 1 to 3, and also buffers and reagents suitable for constituting a reaction medium which allows the formation of an antigen/antibody complex, and/or means for detecting said antigen/antibody complex.

8. The use of a citrullinated polypeptide as claimed in any one of claims 1 to 3, for producing a medicinal product.

9. The use as claimed in claim 8, characterized in that said medicinal product is intended to neutralize the autoimmune response associated with RA.

10. A pharmaceutical composition, characterized in that it contains, as active principle, at least one citrullinated polypeptide as claimed in any one of claims 1 to 3.

ABSTRACT

The invention concerns citrulline polypeptide derived from fibrin useful for diagnosing or treating rheumatoid arthritis.

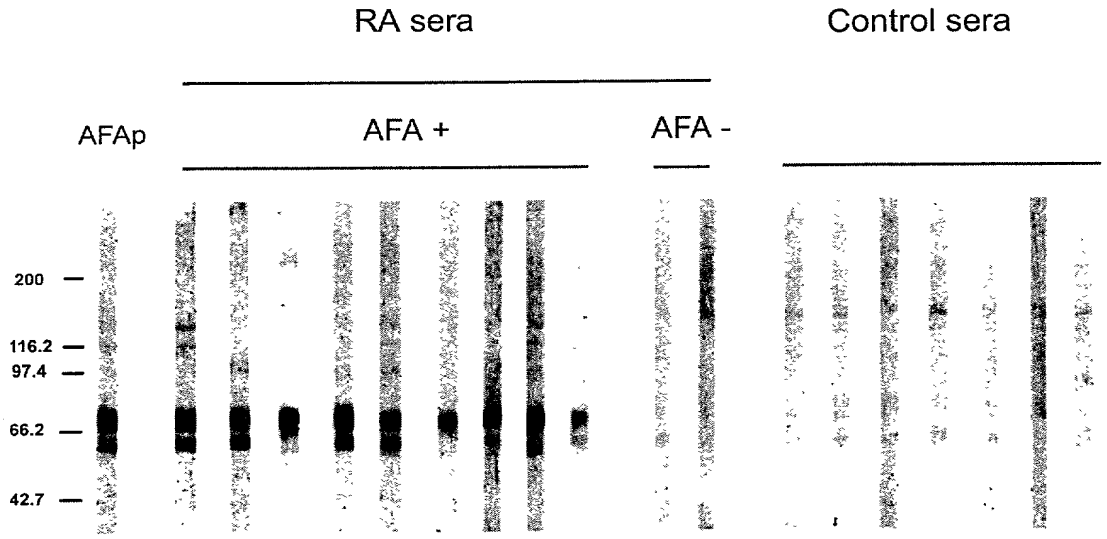

Figure 1

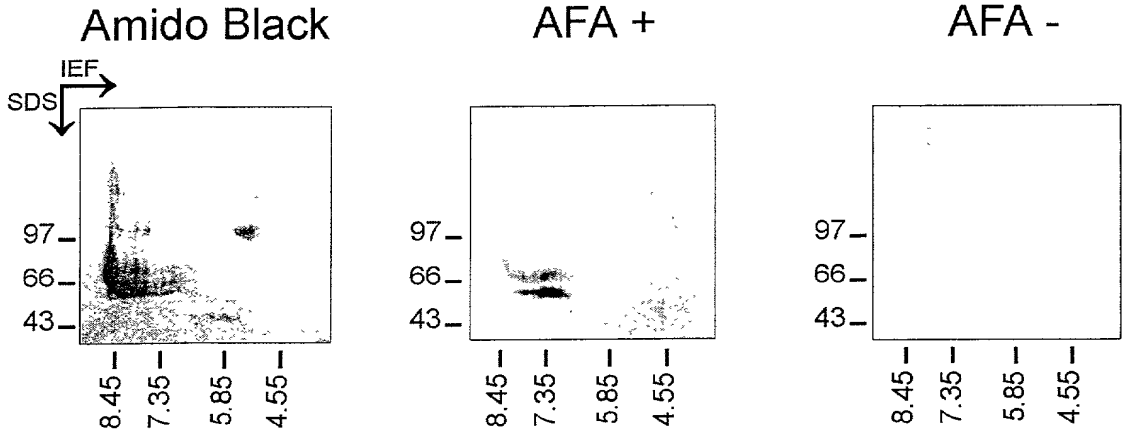

Figure 2

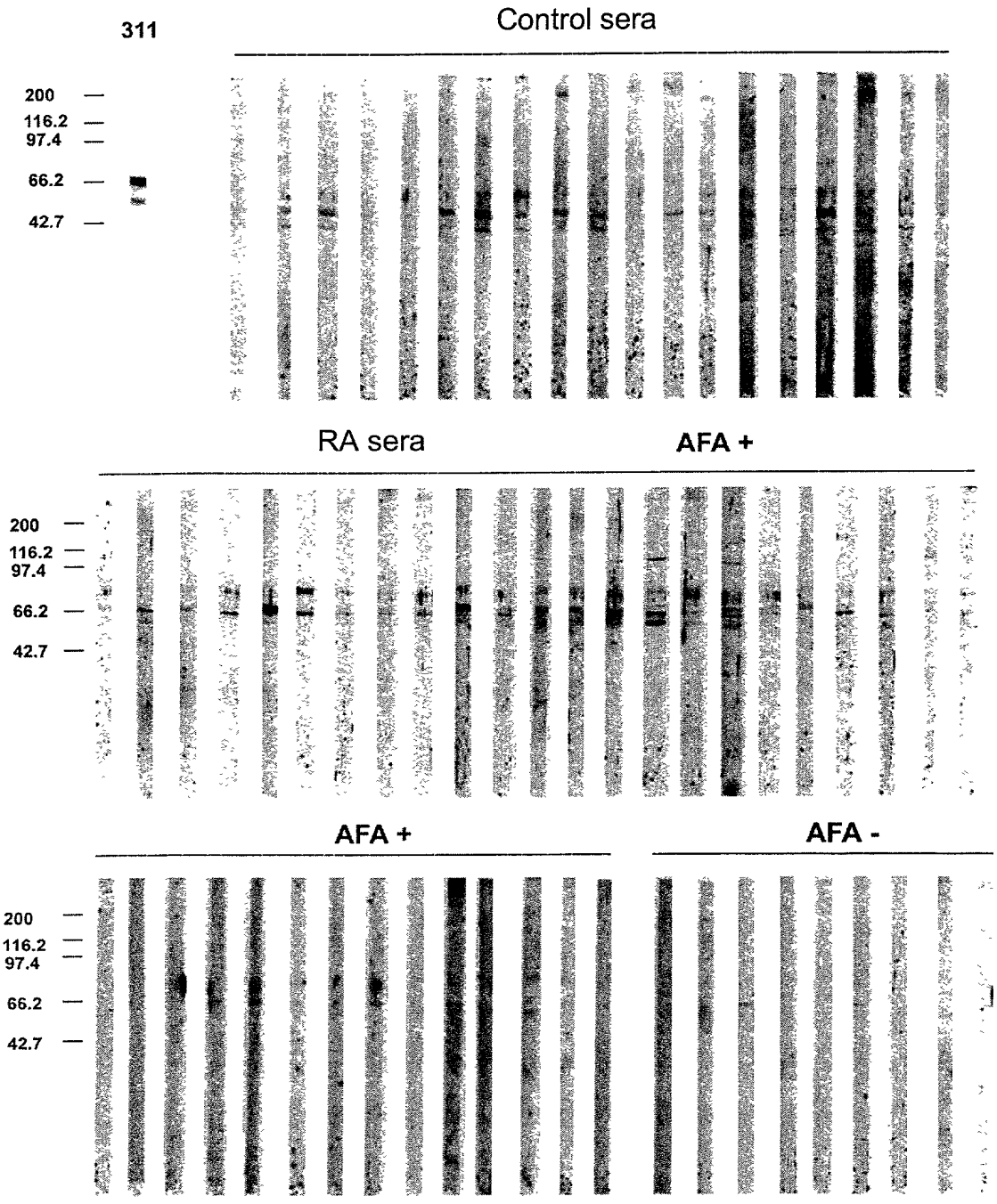
311
Control sera
200
116.2
97.4
66.2
42.7
RA sera
AFA +
200
116.2
97.4
66.2
42.7
AFA +
AFA -
200
116.2
97.4
66.2
42.7

Figure 3A

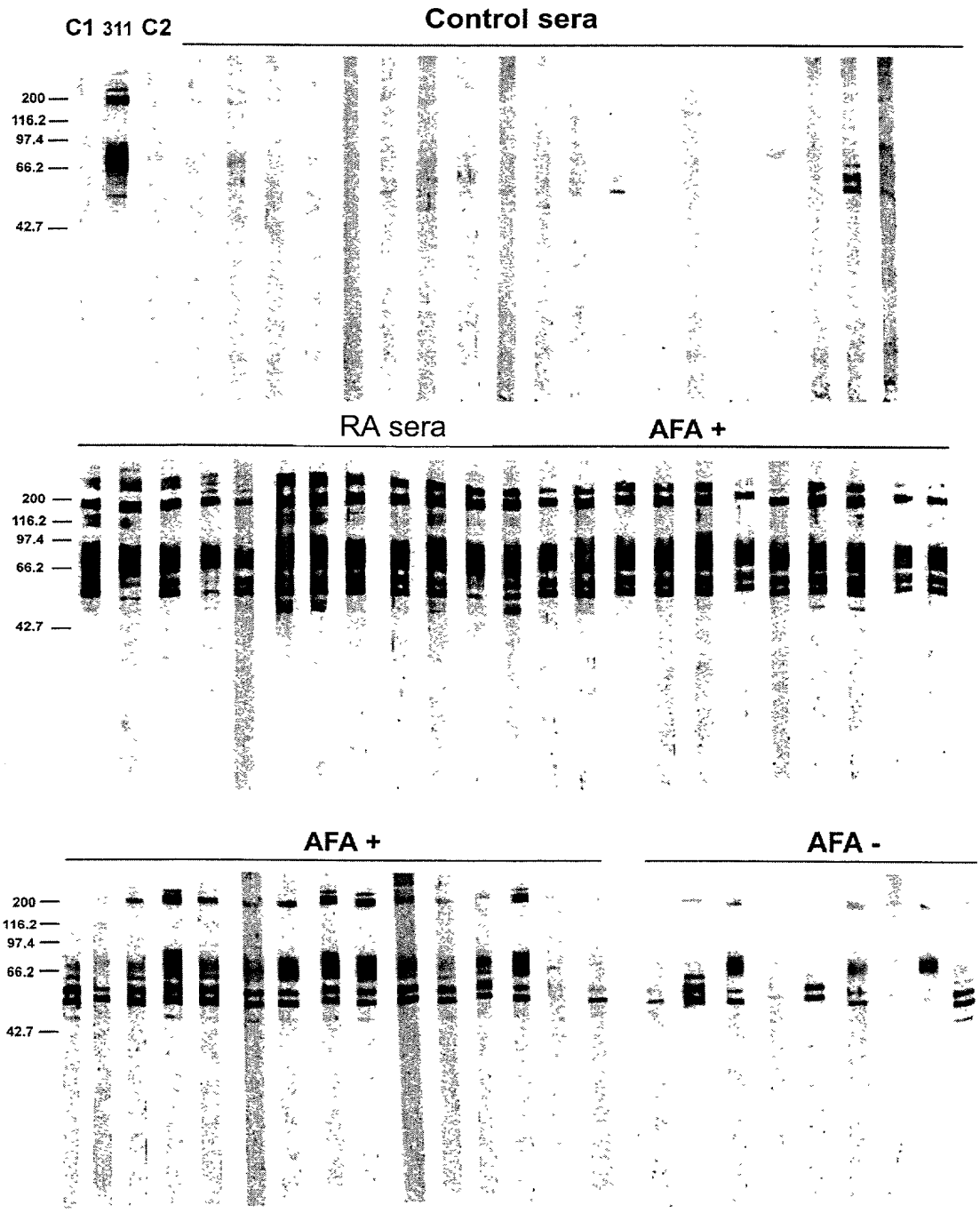

Figure 3B

Declaration and Power of Attorney for Patent Application
Déclaration et Pouvoirs pour Demande de Brevet
French Language Declaration

En tant l'inventeur nommé ci-après, je déclare par le présent acte que :

Mon domicile, mon adresse postale et ma nationalité sont ceux figurant ci-dessous à côté de mon nom.

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) de l'objet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée

et dont la description est fournie ci-joint à moins

☐ ci-joint

☐ a été déposée le

sous le numéro de demande des Etats-Unis ou le numéro de demande international PCT

et modifiée le

(le cas échéant).

Je déclare par le présent acte avoir passé en revue et compris le contenu de la description ci-dessus, revendications comprises, telles que modifiées par toute modification dont il aura été fait références ci-dessus.

Je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations.

As a below named inventor, I hereby declare that :

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed an for which a patent is sought on the invention entitled

FIBRIN CITRULLINE DERIVATIVES AND THEIR USE FOR DIAGNOSING OR TREATING RHEUMATOID ARTHRITIS

the specification of which :

☐ is attached hereto.

☒ was filed on **December 31, 2001**

as United States Application Number or PCT International Application Number.
10/019,439

and was amended on

(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations,§ 1.56.

French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119(a)-(d) ou § 365(b) du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur ou, en vertu du Titre 35, § 365(a) du même Code, sur toute demande internationale PCT désignant au moins un pays autre que les Etats-Unis et figurant ci-dessous et, en cochant la case, j'ai aussi indiqué ci-dessous toute demande étrangère de brevet, tout certificat d'inventeur ou toute demande internationale PCT ayant date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign application(s)
Demande(s) de brevet antérieure(s) dans un autre pays.

(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Anné de dépôt)	Priority claimed Droit de priorité revendiqué ☒ Yes / Oui	☐ No / Non
99/08470	**FRANCE**	**01/07/1999**		
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Anné de dépôt)	☐ Yes / Oui	☐ No / Non

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 119(e) du Code des Etats-Unis, de toute demande de brevet provisoire effectuée aux Etats-Unis et figurant ci-dessous.

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis, ou en vertu du Titre 35, § 365© du même Code, de toute demande internationale PCT désignant les Etats-Unis et figurant ci-dessous et, dans la mesure où l'objet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande antérieure américaine ou internationale PCT, en vertu des dispositions du premier paragraphe du Titre 35, § 112 du code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la demande antérieure et la date de dépôt de la demande nationale ou internationale PCT de la présente demande :

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)	(Status) (patented, pending,abandoned) (Statut) (breveté, en cours d'examen, abandonné)
(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)	(Status) (patented, pending,abandoned) (Statut) (breveté, en cours d'examen, abandonné)

Je déclare que par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique ;et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la section 1001 du Titre 18 du Code de Etats-Unis, et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hebery declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true ; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

French Language Declaration

POUVOIRS : En tant que l'inventeur cité, je désigne par la présente l'(les) avocats(s) et/ou agent(s) suivant(s) pour qu'ils poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire s'y rapportant avec l'Office des brevets et des marquees : (mentionner le nom et le numéro d'enregistrement).

POWER OF ATTORNEY : As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to persecute this application and transact all bussiness in the Patent and Trademark Office connected therewith : (list name and registration number)

Norman F.Oblon, Reg. No. 24,618 ; Marvin J. Spivak, Reg. ~~No. 24,913 ;~~ C. Irvin McClelland, Reg. No. 21,124 ; ~~G~~regory J. Maier, Reg.No. ~~25,599 ; A~~rthur I. Neustadt, Reg. No. 24,854 ; Richard D. Kelly, Reg. No. 27,757 ; James D. Hamilton, Reg. No. ~~28,421 ;~~ Eckhard H. Kuesters, Reg. No. 28,870 ; Robert T. Pous, Reg. No. 29,099 ; ~~C~~harles L. Gholz, Reg. No.26,395 ; ~~W~~illiam E. Beaumont, Reg. No. ~~30,996 ;~~ Jean-Paul Lavalleye, Reg. No.31,451 ; Stephen G. Baxter, Reg. No. 34,884 ; Richard L. Treanor, Reg. No.36,379 ; Stephen P. Weihrouch, Reg. No. 32,829 ; John T. Goolkasian, Reg. No. 26,142 ; Richard L. Cinn, Reg. No. 34,305 ; ~~S~~tephen E. Lipman, Reg. ~~No. 30,011~~ ; Carl E. Shlier, Reg. No. ~~34,426 ;~~ James J. Kubaski, Reg. No. 34,648 ; Richard A. Neifeld, Reg. No. 35,299 ; J. Dereck Mason, Reg. No. 35,270 ; Surinder Sachar, Reg. No. 34,423 ; Christina M. Gadiano, Reg. No. ~~37,628 ;~~ Jeffrey B. McIntyre, Reg. No. ~~36,867 ;~~ William T. Enos, Reg. No. 33,128 ; Michael E. McCabe, Jr., Reg. No. 37,182 ; Bradley D. Lytle, Reg. No. 40,073 ; and Michael R. asey, Reg. No. 40,294, with full powers of substitution and revocation.

29

Addresser toute correspondance à :

Send Correspondence to :

OBLON, SPIVAK, McCLELLAND, MAIER & NEUSTADT, P.C.
~~FOURTH FLOOR~~
1755 ~~JEFFERSON DAVIS HIGHWAY~~
ARLINGTON, VIRGINIA 22202 U.S.A.

Adresser tout appel téléphonique à :
(nom et numéro de téléphone)

Direct Telephone calls to : (name and telephone number)

(703) 413-3000

1-10

Nom complete de l'unique ou premier inventeur **SERRE Guy**	Full name of sole or first inventor
Signature de l'inventeur G. SERRE — Date 12.02.2002	Inventor's signature G. SERRE — Date February 12th, 2002
Domicile 31100 TOULOUSE (FR)	Residence
Nationalité Française	Citizenship
Adresse Postale Résidence du Lac, Appartement 46, 10, Avenue Winston churchill 31100 TOULOUSE (FR)	Post Office Address
Nom complete du second co-inventeur, le cas echeant **SEBBAG Mireille**	Full name of second joint inventor, if any
Signature de l'inventeur M. SEBBAG — Date 12.02.2002	Second inventor's signature M. SEBBAG — Date February 12th, 2002
Domicile 31500 TOULOUSE (FR)	Residence
Nationalité Française	Citizenship
Adresse Postale 3, Rue A. Frédeau 31500 TOULOUSE (FR)	Post Office Address

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)